INVESTIGATION OF NOVEL THERAPEUTIC POTENTIALS OF *H. ISORA* AND DEVELOPMENT OF HERBAL NOVEL FORMULATION

Deependra Soni[1,3], Anshita Gupta Soni[2,] Chanchal Deep Kaur[2]

1.Rungta College of Pharmaceutical Sciences and Research, Bhilai, Chhattisgarh, India
2. Rungta College of Pharmaceutical Sciences and Research, Raipur, Chhattisgarh, India
3 Faculty of Pharmacy, MATS University Campus, Aarang, Raipur, Chhattisgarh, India
Email: dsresearch24@gmail.com, anshita1912@gmail.com

ABSTRACT

The purpose of this study was to investigate the in vitro drug release profile and skin retention of *H. Isora* extract-loaded phytophospholipid complexes. FE2 (ethanolic extract) and FH4 (hydroalcoholic extract) were optimized formulations. The drug release profiles of FE2 and FH4 were examined in phosphate-buffered saline (PBS) at 37°C under physiological and acidic pH conditions (5.6), finding that the cumulative release of FE2 was approximately 90% after 6 hours. In the first two hours, approximately 35% of the drug was released, followed by a slower, sustained release phase. A stability study was carried out according to ICH guidelines (Q1AR2) in which formulations were stored at room temperature and at accelerated conditions (25oC, 60% RH; 40oC, 75%) for six months. Stability of formulations was confirmed by determining particle size, zeta potential, and entrapment efficiency at defined intervals. An assessment of skin retention of the formulations over time was conducted using abdominal goat skin. An incubation period of 32°C was established for fresh goat skin samples treated with phytophospholipid complexes formulations. For determining the amount of drug retained, the formulations were removed from the skin at intervals and analyzed with HPLC. According to the retention profiles, both the FE2 and FH4 formulations achieved effective skin retention, suggesting that prolonging therapeutic action could be possible with these formulations. In conclusion, these studies confirm the efficacy and stability of *H. isora* extract-loaded phytophospholipid complexesthat can be used for advanced drug delivery applications.

KEYWORDS: *Helicteres isora*, Phytophospholipid complexes, Anti- inflammatory activity, phytophospholipid complex, skin permeation

1. INTRODUCTION

It is known as the Indian screw tree or *Helicteres isora* (*H. isora*). It has traditionally been used in various medicinal systems across Asia for its supposed health benefits(1). It is known for its diverse therapeutic applications, including the treatment of gastrointestinal disorders, diabetes, infections, and inflammation. The plant's widespread use in folk medicine underscores the need for scientific validation of its medicinal properties(2). Despite its extensive use in traditional medicine, there is a paucity of comprehensive scientific research on *H. isora*. The active compounds responsible for its therapeutic effects and their mechanisms of action remain largely unexplored(3). With the increasing interest in natural and alternative therapies, it is essential to scientifically investigate the therapeutic potentials of *H. isora*. Additionally, developing a novel herbal formulation based on these findings could offer a natural, safe, and effective alternative to conventional therapies.

An unpleasant sensory and emotional experience associated with actual or potential tissue damage, pain is a multifaceted and complex experience(4). Protecting individuals against further injury, it alerts them to harmful stimuli and prompts them to take action. Pain can be classified into various types, such as acute or chronic, nociceptive or neuropathic, each with distinct underlying mechanisms and treatment approaches. Despite advancements in medical science, pain management remains a significant challenge, with many patients suffering from inadequate relief. Understanding the intricacies of pain is essential for developing effective therapeutic strategies and improving patient quality of life(5).

Herbal medicine, an integral part of traditional healthcare systems worldwide, has gained renewed interest in recent years due to its natural origin and perceived safety(6). The growing demand for natural remedies has driven research into various medicinal plants to discover and validate their therapeutic potentials(7). There are many medicinal properties associated with Helicteres isora (*H. isora*), including its anti-inflammatory, antimicrobial, and antioxidant properties. This study seeks to bridge the gap between traditional knowledge and modern scientific research by providing a detailed investigation of H. isora's medicinal properties(8). By identifying and characterizing the bioactive compounds, the study aims to validate the traditional uses of *H. isora* and uncover new therapeutic applications(9). Furthermore, the development of a novel herbal formulation could

contribute to the growing market for natural health products, offering potential benefits for public health and wellness.

2. MATERIALS AND METHODS

2.1. Materials

Helicteres isora , Malvaceae was collected from the local area of Balodabazar distict (ganiyari ,kasdol pin code 492112). Plant identification and authentication were performed by Dr. Praveen Kumar Joshi (HOD and Professor) of the Govt. Ayurvedic College Raipur, C.G. Rosmarinic acid (bioactive) was obtained from Sami Labs, India. Sample of Caffeic acid was purchased from Biomolecules India. All other chemicals used were of analytical grade.

2.2. Extraction and characterization of *Helicteres isora* fruit

We dried the fruits of the plant species collected under shade for four weeks. A commercial laboratory blender was used to grind the dried plant material into a fine powder. A Soxhlet extractor was used to extract the powder with ethanol for about 8-9 hours at 30°C, and the extract was collected and dried using an evaporator at 30-35°C, and crude residue was collected. It was kept in a refrigerator at 5 °C in a well-closed glass container.

2.3.Preparation of phytophospholipid complexes

A modified solvent evaporation method was used to prepare phytophospholipid complexes. With the help of a solvent evaporation technique, phytophospholipid complexes complexes were prepared from extracts of *H.isora* of a 1:1 molar ratio in trial batches. Specifically, 100 ml of *H.isora* extract and soya lecithin were placed into a 25 ml round bottom flask and refluxed for 2 hours with 20 ml of acetone. To obtain the precipitate, the mixture was concentrated to 5-10 ml and filtered. Stored at room temperature, dried precipitate phytophospholipid complexes complex was placed in amber-colored glass bottles.

2.4.Preparation of Herbal Extract Loaded Phytophospholipid complexes

The different phytophospholipid complexes complexes of *H.isora ethanolic and hydroalcoholic* extracts containing molar ratio of 1:1, 1:2, 2:1, 2:2, 3.1,3.2 of both *H.isora* extracts and soya lecithin were prepared by the solvent evaporation technique as mentioned in Table No. 1 and 2 .

Table. No.1: Showing Molar ratio of Drug and Phospholipid for different formulations of ethanolic extracts of *H.Isora* (FE1 to FE6)

Formulation Code	Phytophospholipid complexes (Molar ratio)	Drug	Phospholipid
FE1	1:1	H.Isora ethanolic extract	Soya lecithin
FE2	1:2	H.isora ethanolic extract	Soya lecithin
FE3	2:1	H.isora ethanolic extract	Soya lecithin
FE4	2:2	H.isora ethanolic extract	Soya lecithin
FE5	3.1	H.Isora ethanolic extract	Soya lecithin
FE6	3.2	H.isora ethanolic extract	Soya lecithin

Table. No.2: Showing Molar ratio of Drug and Phospholipid for different formulations of hydroalcholic extracts of *H.Isora* (FHE1 to FHE6)

Formulation Code	Phytophospholipid complexes (Molar ratio)	Drug	Phospholipid
FH1	1:1	H.Isora Hydroalcoholic extract	Soya lecithin
FH2	1:2	H.isora Hydroalcoholic extract	Soya lecithin
FH3	2:1	H.isora Hydroalcoholic extract	Soya lecithin
FH4	2:2	H.Isora Hydroalcoholic extract	Soya lecithin
FH5	3.1	H.Isora Hydroalcoholic extract	Soya lecithin
FH6	3.2	H.isora Hydroalcoholic extract	Soya lecithin

2.5.Characterization of phytophospholipid complexes

The prepared phytophospholipid complexeswere examined for various physical properties like (size, size distribution, zeta potential), encapsulation efficiency, and morphology using scanning electron microscopy (SEM)(10).

2.5.1. Size and Zeta Potential of Phytophospholipid complexes

Dynamic light scattering (DLS) technique was used to measure particle size, polydispersity index (PDI), and zeta potential in *H. isora* phytophospholipid complexes containing phytophospholipid complexess. A Malvern Zetasizer Nano-ZS (ZEN3600, Malvern Instrument Ltd., Malvern, UK) with a laser at 633 nm was used to measure these parameters A dilution of the samples was performed with ultrapure water of 1 - 2 mL in order to get the best scattering intensity. Using a standard operating method, we measured the intensity of scattered light at an angle of 173°. An average of three runs is reported for each sample's size average of the intensity distribution (11). The zeta potential of the sample was determined using ELS in the same measuring cuvette after the electrode Universal Dip Cell (ZEN1002) had been immersed in the sample after determining the size. ZetaSizer Software 7.12 was used to analyze the results shown in table no. 3 and 4 (Malvern Instruments).

2.5.2. Determination of entrapment efficiency:

H.isora extracts loaded with phospholipid complex (HEPC) were diluted 1-fold with 10 ml of ethanol, centrifuged at 18,000 revolutions per minute at -4oC for 1/2 hour, and subsequently analyzed by UV Spectroscopy. In order to determine the amount of free *H.isora extracts*, the supernatant was isolated and UV/Vis spectroscopy at 280 nm was performed. A 10 ml volume of methanol was adjusted to 0.1 ml of hydrogen isora extracts phospholipid suspension to determine the quantity of H. isora extracts. The results are shown in table nos. 3 and 4. This formula was used to calculate entrapment efficiency:

$$\textbf{Entrapment efficiency (\%)} = \frac{\text{(Total amount of drug)} - \text{(amount of free drug)}}{\text{(Total amount of drug)}} \times 100$$

2.5.2. Percentage yield

To determine the percentage yield of a *H.isora* containing phytophospholipid complexes, first extract the desired phytoconstituents from the plant material using a suitable solvent, then filter and concentrate the extract. The phospholipid was dissolved in an appropriate solvent and mixed with the concentrated plant extract, heating and stirring the mixture to form the phytophospholipid complexes complex. The solvent was removed using a rotary evaporator, then dry the resulting phytophospholipid complexes complex in a desiccator. Result show in table no. 3 and 4 (12). Weigh the dried phytophospholipid complexes complex and calculate the percentage yield using the formula:

$$\text{Percentage Yield complexes Complex)} = \frac{\text{(Final Weight of Phytophospholipid}}{\text{(Initial Weight of Plant Extract and Phospholipid)}} \times 100$$

2.5.4. FTIR (Fourier Transform Infrared) spectroscopy

H.isora extract phytosomal suspensions were examined with FTIR spectra using an FTIR microscope to see how the extract interacts with phospholipids. Using the KBr disc technique, the infrared spectrum of the test samples was determined. In this study, FTIR measurements were performed in the range of 4000 to 400 cm-1. Fruit extracts of *H.isora*, lipoids P 30, a mixture of fruit extracts and lipoids P 30, and freeze-dried phytophospholipid complexeswere analyzed. The Fourier Transform Infrared Spectroscopic (FTIR) study was conducted on pure RA, PC, physical mixture (PM), FE2 and FH4 in the FTIR spectrophotometer (a Shimadzu Corporation product).

2.5.5. XRD (X-Ray Diffraction)

The X-ray powder diffraction method is used to investigate material structural characteristics. Powders and polycrystalline materials will scatter X-rays differently, depending on their crystalline structure. The scattered pattern of a material allows it to be identified as such(13). The powder diffraction process is a bulk sample procedure for identifying solids and heterogeneous samples as well as monitoring solid-state reactions. Solid-state scientists can use amorphous patterns to determine the structure of solids. They will also scatter X-rays(14).

2.5.6. I*n vitro* drug release profile

In vitro drug release profiles of *H. isora* ethanolic extract loaded phytophospholipid complexes(FE1 to FE5) and hydroalcoholic extract loaded phytophospholipid complexes(FH1 to FH5) were analyzed at 37°C in phosphate buffered saline (PBS). By dispersing phytophospholipid complexesin PBS containing microcentrifuge tubes at physiological pH and acidic pH (5.6), the study was performed at physiological pH and acidic pH (5.6).

2.5.7. Stability studies

Stability studies of optimized *H. isora* ethanolic extract loaded phytophospholipid complexes(FE2) and H. isora hydroalcoholic extract loaded phytophospholipid complexes(FH4) were conducted according to ICH guidelines (Q1AR2). The formulations were stored under different conditions: room temperature, accelerated temperature (25±2ºC, 60±5% RH), and (40±2ºC, 75±5% RH) for 6 months. At specified intervals, samples were analyzed in triplicates for average particle size, zeta potential, and entrapment efficiency to assess their stability.

2.6.Preparation of Phytophospholipid Complex loaded Gel Formulations

An appropriate amount of methyl and propyl paraben was dissolved in 10 ml of distilled water, then Carbopol® 934P was mixed continuously while the mixture was mixed. A solution of RA/FE2/FH4 (Pure rosmarinic acid/ Ethanolic extract/Hydroalcholic extract) in 0.1 ml of ethanol was prepared in another beaker and added to the Carbopol dispersion to prepare RA/FE2/FH4 in 0.05 ml of ethanol. The dispersion was also formulated with PEG 400. As a gelling agent, triethanolamine was added to the dispersion. For further experiments, gels were filled in Al-tubes and stored at room temperature. A variety of excipients and solvents were used to prepare gels for phytophospholipid complexes and pure drugs, which are summarized in Table No. 3.

Table No. 3 The gels of the phytophospholipid complexes and pure drug were prepared by using different excipients and solvents as mentioned in below:

Name of Ingredients	**Formulation of Drug Gel**		**Formulation of Complex Gel of 1:1**		**Formulation of Complex Gel of 1:2**	
	RA_G1	**RA_G2**	**FE_G1**	**FE_G2**	**FH_G1**	**FH_G2**

Carbopol® 934P	1%	1%	1%	1%	1%	1%
PEG 400	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%
Pure Drug(RA)/ FE2/FH4	1%	2%	1%	2%	1%	2%
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Methyl Paraben	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Propyl Paraben	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Ethanol	1%	1%	1%	1%	1%	1%
Distilled Water	q.s	q.s	q.s.	q.s	q.s	q.s

2.7. Evaluation of Phytophospholipid Complex loaded Gel Formulations

2.7.1. Aspects of physical appearance

There was a check on physical parameters such as color and appearance(15).

2.7.2. pH measurement

Prepared gel formulation pH was determined by using a digital pH meter. Disperse 1 gm of gel in 10 ml of distilled water and leave for 2 hours. In three separate measurements, the pH of formulation was determined, and the average values were reported(15). The pH of the gel formulation is shown in table no. 7.

2.7.3. Spreadability

Spreadability was determined by placing 0.5 g of gel within a circle of 1 cm diameter premarked on a glass plate, over which another glass plate was placed. The upper glass plate was allowed to rest for five minutes with a weight of 1000g. It was noted that the diameter increased due to the gel spreading. With three samples of the gel, evaluations were conducted in triplicate(16).

2.7.4. Viscosity

The viscosities were measured using a Brookfield (DV-III) viscometer. For it a spindle (number 74) was attached to the container after each gel was poured into it. At 25°C and 50-250 rpm, viscosities were measured(17).

2.7.5. Uniformity

The uniformity of all gel formulations was checked by visual inspection after the gels were placed into the containers. A test was performed to determine if they contained or appeared to contain aggregates(18). Table 7 shows the uniformity of gel formulations.

2.7.6. TEM (Transmission electron microscope)

A transmission electron microscope (TEM) (Morgagni 268-D) was used at an accelerating voltage of 100 kV to monitor the morphology of all phytophospholipid complex-loaded gel formulations. Gel samples were dissolved in distilled water and a drop of the solution was placed on a carbon-coated copper grid to form a thin film, and negatively stained using phosphotungstic acid 1% w/v. After the grid was allowed to air dry, samples were viewed and photographed. (Figure No. 7).

2.7.7. *Ex-vivo* skin permeation tests

2.7.7.1. Skin penetration study of different formulations by Confocal Laser Microscopy [CLSM]

All the formulations were studied via confocal laser microscopy on goat skin *in vitro* to determine their skin penetration effects. To evaluate the penetration ability of phospholipid complexes into skin, goat skin was used since it shares anatomical and physiological similarities with human skin. Rhodamine 123 was used to assess the skin penetration of all six formulations. Throughout the study, homogeneous and non-occlusive applications of the test samples and probe containing 0.03% rhodamine were applied to the skin. We conducted the experiments using Franz diffusion cells and phosphate buffer pH 5.5 solution in the receiver chamber. With phosphate buffer, the skin was washed after 24 hours. A sharp blade was used to take a perpendicular rectangular piece from the site of drug application after the skin was rapidly frozen by liquid nitrogen. The Tissue frozen medium gel was used to fix this tissue on the sample holder (Gung,Leica, Germany).

The skin was sectioned with a cryo microtome (Leica, Germany) in perpendicular sections (dermis to horny layer) of (250 m) thickness. Using a Leica DMIRE2 confocal laser scanning microscope

(Germany) with a Leica TCS SP2 fluorescence microscope, the full skin thickness was optically scanned at intervals of 15-30nm using the Z-axis.

2.7.7.2.Skin Retention Study

The skin retention study was conducted using abdominal goat skin to evaluate the retention of H. isora ethanolic extract loaded phytophospholipid complexes(FE2) and H. isora hydroalcoholic extract loaded phytophospholipid complexes(FH4). Fresh goat skin samples were cleaned, and subcutaneous fat was removed before being stored in isotonic saline until use. A specified amount of each formulation was applied evenly to the skin surface, with a control formulation used for comparison. The samples were incubated at 32±1°C to mimic physiological conditions, and the formulations were removed at predetermined intervals (1, 2, 4, 6, 8, 12, and 24 hours) using a suitable solvent. The skin samples were then cut, homogenized in a solvent, and centrifuged to extract the retained formulation. The extracts were analyzed using HPLC to quantify the amount of drug retained in the skin, providing a retention profile over time.

2.7.7.3.**Glutamate-induced paw licking and edema test**

A concentration of 20 mol glutamate was injected in the sub-plantar area of the right hind paw of mice pretreated with saline, pH 7.4. A vehicle, test sample, and diclofenac sodium were given to mice 15 minutes before glutamate injection. An instrument called a vernier calliper was used to measure the thickness of the right hind paws of mice before glutamate challenge. During the 15 minutes following glutamate challenge, licking was measured as an indicator of nociception. To calculate edema in mm, the thickness of the right hind paw was measured again, and the formula follows:

Δ = (paw thickness after treatment – paw thickness before treatment).

3. RESULT AND DISCUSSION

H. isora is a plant that has been traditionally used in Ayurvedic medicine for its anti-inflammatory, antioxidant, and anti-diabetic properties. Recent studies have shown that *H. isora* contains bioactive compounds such as flavonoids, alkaloids, and tannins, which have potential therapeutic effects on various diseases. In light of these findings, there was a need to further investigate the novel therapeutic potentials of *H.isora* and develop herbal formulations for clinical applications. Through rigorous literature review, it was found that the fruits of H.isora is less studied as

compared to its leaves part but have higher therapeutic potential. To begin the investigation, two different solvent system were used for extraction (ethanolic and hydroalcholic) and followed by preparation of phytophospholipid complexesof both extracts. First the powdered drug were extracted with ethanol as well as with ethanol: water (60:40) for about 8-9 hours at 30°C and the extracts were collected and dried using an evaporator at 30-35°C followed by collections of crude residues was collected which were kept in a refrigerator at 5 °C in a well-closed glass containers. The product yield for 100 gm of *H.isora* ethanolic and hydroalcholic extract were nfound to be 3.58gm±0.023 and 5.47 gm±0.51 respectively. The phyto-chemical screening revealed the presence of alkaloid , glycosides, flavonoids, tannin, saponins and cardiac glycosides are present in ethanolic extract while alkaloids were found to be absent in hydroalcholic extract.

3.1. **Characterization of the Prepared Phytosomal Formulations**:

3.1.1. Zeta Potential and Particle Size Analysis:

As the objective of the research was to enhance the drug delivery of *H.isora* extracts, the prepared extracts were subjected to modified solvent evaporation method for development of extract loaded phytosomal preparations. Phytophospholipid complexes complex of both the extracts of *H.isora* in 1:1 molar ratio trial batches were prepared by using modified solvent evaporation techniques and were subjected to various characterizations like particle size, zeta potential, drug entrapment and % yield. In the case of *H. isora* phytophospholipid complexess, Dynamic Light Scattering method was used to measure the average particle size and polydispersity index, which is a measure of the distribution of particle sizes in a sample. These properties were measured using a Malvern Zetasizer Nano-ZS (ZEN3600, Malvern Instrument Ltd., Malvern, UK) with a laser at 633 nm. To acquire the optimal scattering intensity, the samples were diluted using 1 to 2 mL of ultrapure water. The intensity of dispersed light at an angle of 173° using a conventional operating procedure was measured. For each sample, the size average of the intensity distribution is provided as an average of three runs (11). After estimating the size of the sample, the electrode Universal Dip Cell (ZEN1002) was submerged in it, and the zeta potential of the sample was calculated using ELS in the same measuring cuvette. The prepared formulations had a range of particle sizes from 125.6 nm to 250.39 nm. Zeta potential measurements indicated values ranging from -19.72mV to -29.05 mV (Figure 1 and Figure 2). The results of various characterization parameters are mentioned below in the Table no. 4 and 5. In general, zeta potential value of ± 30Mv is sufficient for stability

any vesicular formulation. In the present research work the prepared formulations exhibited zeta potential it is -19.72mV to -29.05 mV which means it complies with requirement of zeta potential for stability. Similarly, Here particle sizes were measured in the terms of average particle size diameter and the uniformity was described in the poly dispersity Index (PDI). poly dispersity Index (PDI) was found to be 0.173. A PDI value of 0.1 - 0.3 indicates a fairly narrow size distribution whereas a PDI value greater than 0.5 indicates a very broad distribution. Average particle size of optimized formulations was found to be from 125.6 nm to 250.39 nm

Table No. 4. The qualitative results obtained from *H.isora* ethanolic extract loaded Phytophospholipid complexes(FE)

Formulation code	% Yield	Particle size (nm)	Size Distribution (PDI)	Zeta Potential (mV)	% Entrapment Efficiency
FE1	87.10	176.43±1.28	**0.2±0.6**	**-25±0.5**	80.3 ± 0.5
FE2	83.33	150.22±2.7	**0.17±0.2**	**-28±0.6**	91.3 ± 0.2
FE3	75.09	227.14±2.65	**0.15±0.1**	**-30±0.7**	89.3 ± 0.8
FE4	69.87	230.7±1.56	**0.15±0.23**	**-26±0.2**	91.0 ± 0.2
FE5	70.23	211.32±1.66	**0.23±0.5**	**-29±0.5**	71.1 ± 0.2

Table No. 5. The qualitative results obtained from H.isora hydroalcoholic extract loaded Phytophospholipid complexes(FH)

Formulation code	% Yield	Particle size (nm)	Size Distribution (PDI)	Zeta Potential (mV)	% Entrapment Efficiency
FH1	83.10	150.23±1.45	**0.19±0.7**	**-25±0.5**	72.7 ± 0.5
FH2	77.13	233.43±2.65	**0.18±0.23**	**-28±0.6**	77.5 ± 0.8
FH3	77.89	278.12±2.10	**0.22±0.18**	**-32±0.7**	79.2± 0.17
FH4	**71.98**	**157.44±1.98**	**0.19±0.5**	**-26±0.2**	**83.01 ± 0.7**
FH5	68.41	325.39±2.08	**0.21±0.6**	**-29±0.5**	71.4 ± 0.52

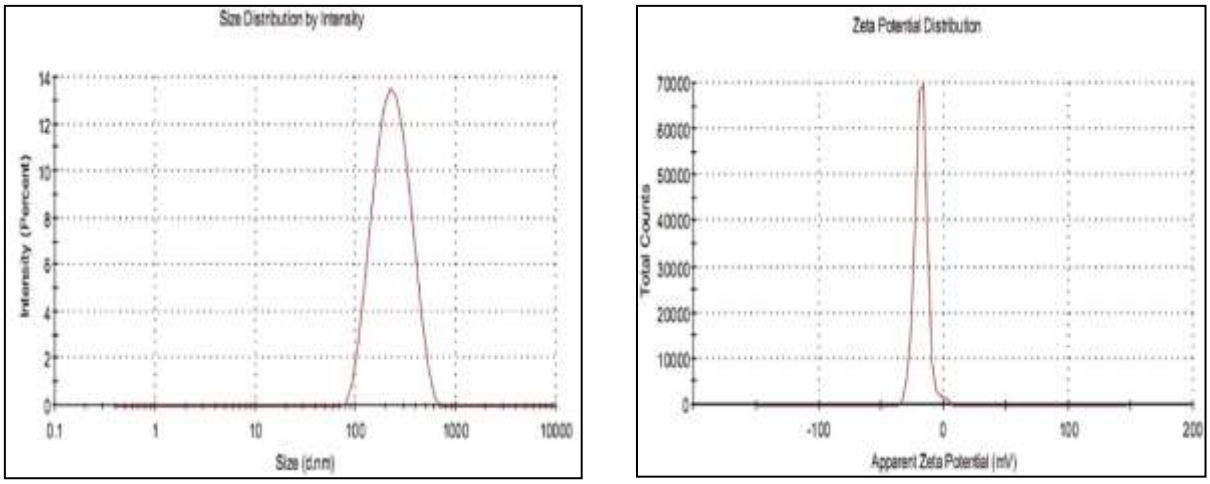


Figure No.1. Figures showing Particle size and Zeta Potential of *H.isora* ethanolic extract loaded Phytophospholipid complexes(FE2)

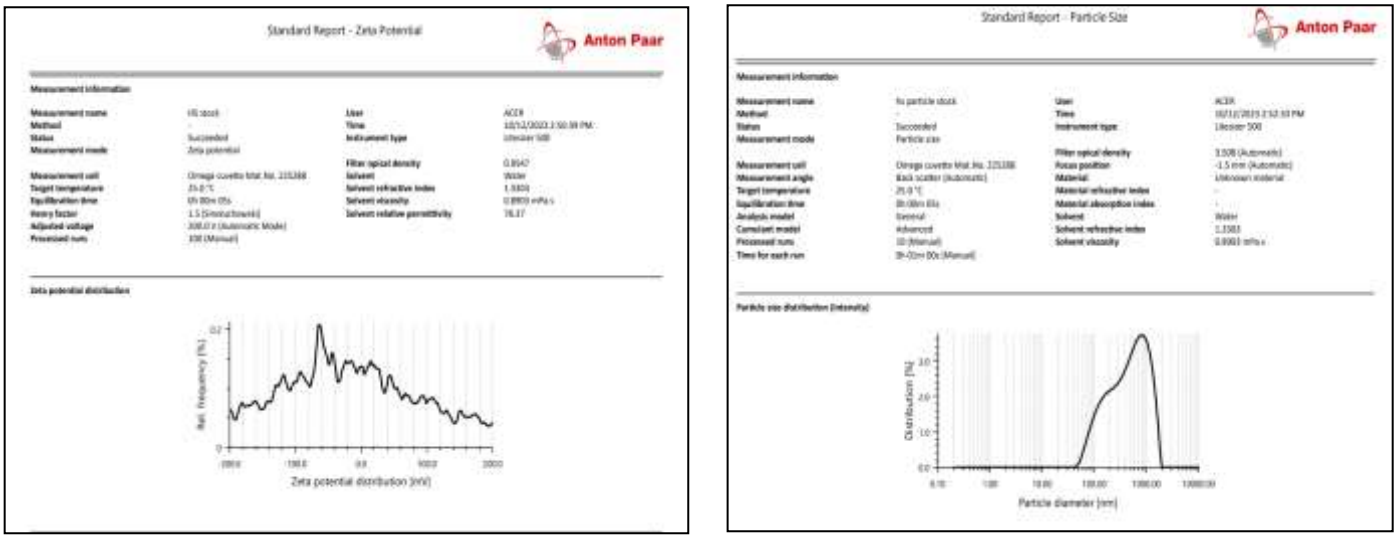


Figure No. 2. Figures showing Particle size and Zeta Potential of *H.isora Hydroalcholic* extract loaded Phytophospholipid complexes(FH4)

3.2. Scanning Electron Microscopy (SEM):

A scanning electron microscope was used to study the morphology of the optimum phytosmes loaded with both the extracts. The particles was found to be not overall sphere in shape which was given in Figure No.3.

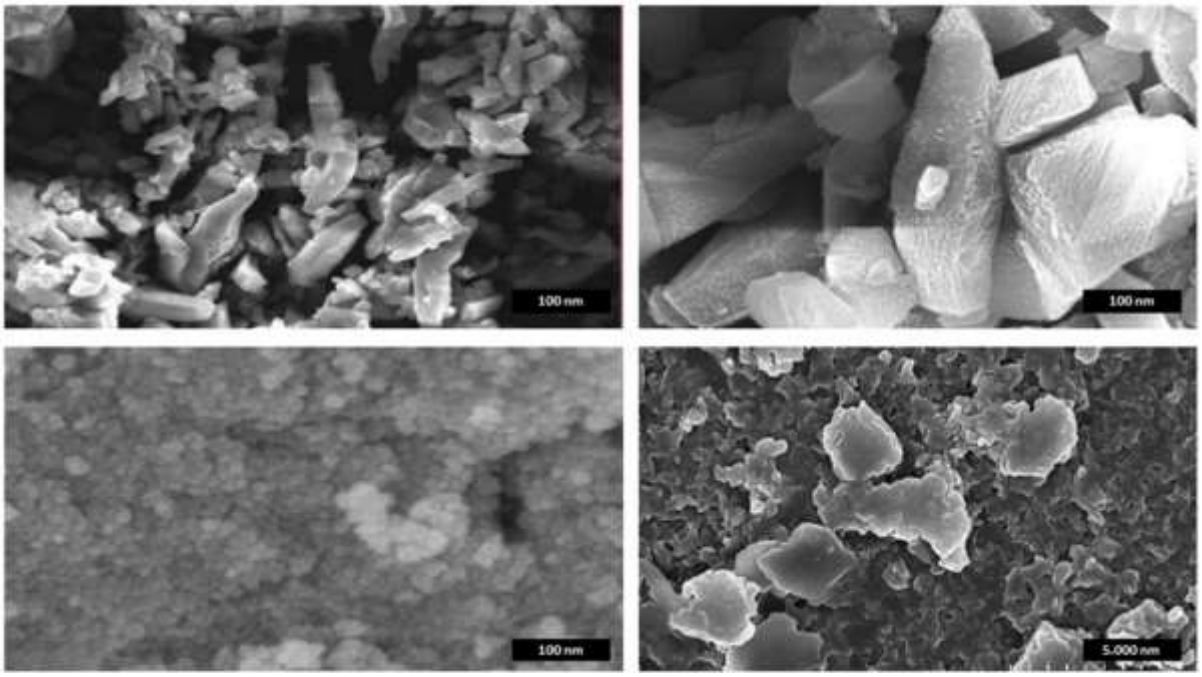


Figure No. 3. Figures showing SEM Images of Optimized *H.isora* ethanolic extract-loaded Phytophospholipid complexes(FE2) and *H.isora* Hydroalcholic extract loaded Phytophospholipid complexes(FH4)

3.4. Fourier Transform Infrared spectroscopy (FTIR) Study

Using an FTIR microscope, FTIR spectra of phytosomal suspensions loaded with *H. isora* extract were analyzed to determine the extract's interaction with phospholipids. The test samples' infrared spectra were ascertained by applying the KBr disc method. FTIR measurements in this investigation were conducted in the 400 cm-1 to 4000 cm-1 range. The Fourier Transform Infrared Spectroscopic (FTIR) study was conducted on pure RA, PC, physical mixture (PM), FE2 and FH4 in the FTIR spectrophotometer (a Shimadzu Corporation product). The results of the FTIR analysis showed that these peaks were also observed in the Physical mixture (PM) spectrum, indicating limited interaction between the phenolic hydroxyl group in RA and the structure of soya lecithin(Figure No. 4). Contrary to the peaks observed in FE2 and FH4, these respective peaks disappeared in FE2, suggesting that complexation caused strong phenolic hydroxyl interactions with soya lecithin. Hydrogen bonds are likely formed between the -OH groups in RA and the -O groups in soya lecithin, leading to the changes.

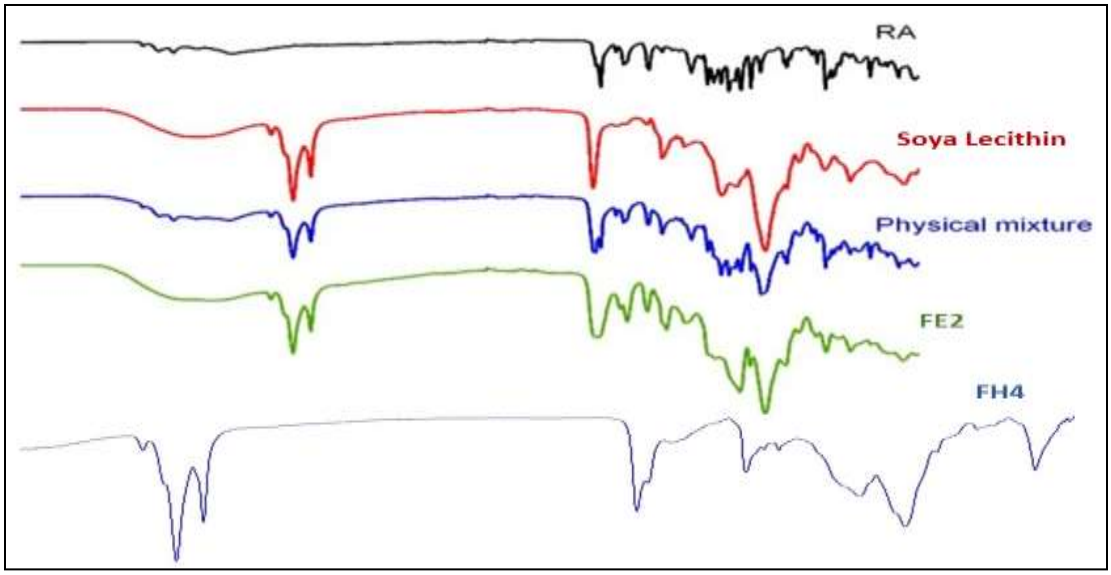


Figure No. 4 FTIR spectra of (A) RA, (B) PC, (C) Physical mixture (D) FE2 and (E) FH4

3.5.**XRD Study**

The structural properties of materials are examined using the X-ray powder diffraction technique. Depending on their crystalline structure, powders and polycrystalline materials will scatter X-rays in different ways. A substance can be recognized as such by its dispersed pattern(13). A sharp peak was seen in the diffraction pattern of unformulated RA, showing a high degree of crystallinity. Amorphous bands were evident in the phospholipids used in this study, indicating that they are not crystalline Amorphous states were evident in the spectra of FE2 and FH4, as reflected by halo bands similar to those of phospholipids. As a result of the amorphous profile, it can be concluded that RA has been complexed to the phospholipid successfully (Figure No.5).

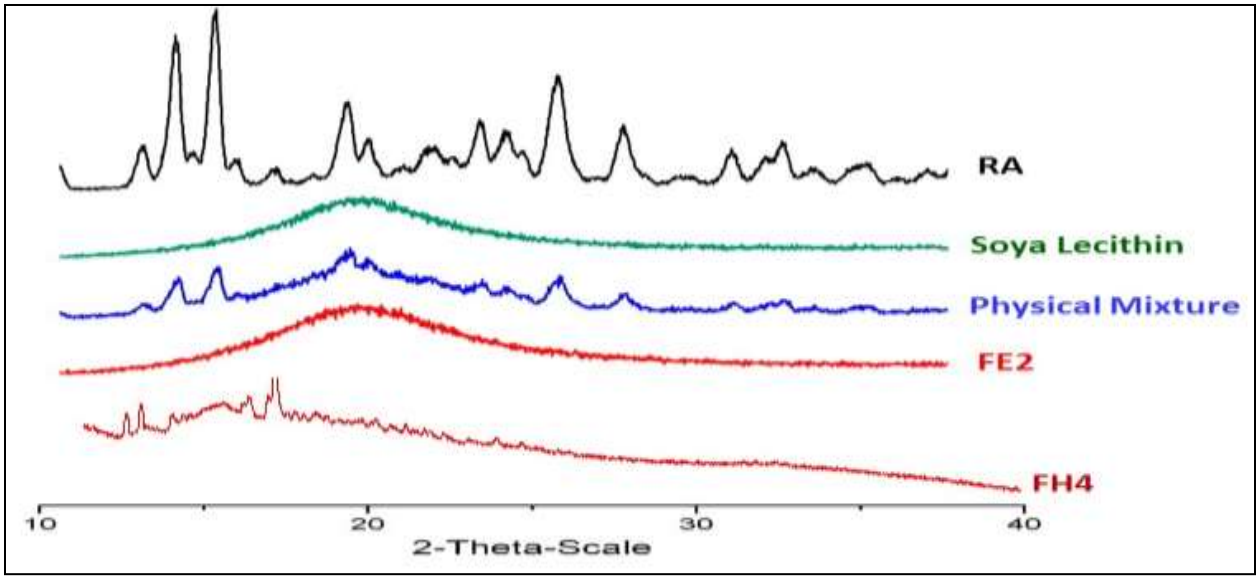


Figure No. 5. XRD diffractogram of Rosmarinic acid (RA), Soyalecithin, Physical mixture,FE1, and FH4

3.6. *In vitro* drug release profile

In vitro drug release profiles of *H. isora* ethanolic extract loaded phytophospholipid complexes(FE1 to FE5) and hydroalcoholic extract loaded phytophospholipid complexes(FH1 to FH5) was analyzed at 37°C in phosphate buffered saline (PBS). By dispersing phytophospholipid complexesin PBS containing microcentrifuge tubes at physiological pH and acidic pH (5.6), the study was performed at physiological pH and acidic pH (5.6). After 6 hours, both pH conditions showed more or less the same drug release profile. The cumulative release in both pH conditions was 90 percent of the initial drug loading. As can be seen in the figure 6, the initial release of the drug at pH 5.6 was 35% in the first two hours. Later stages of the experiment were characterized by a slower and approximately steady state (lag state) with sustained release kinetics.

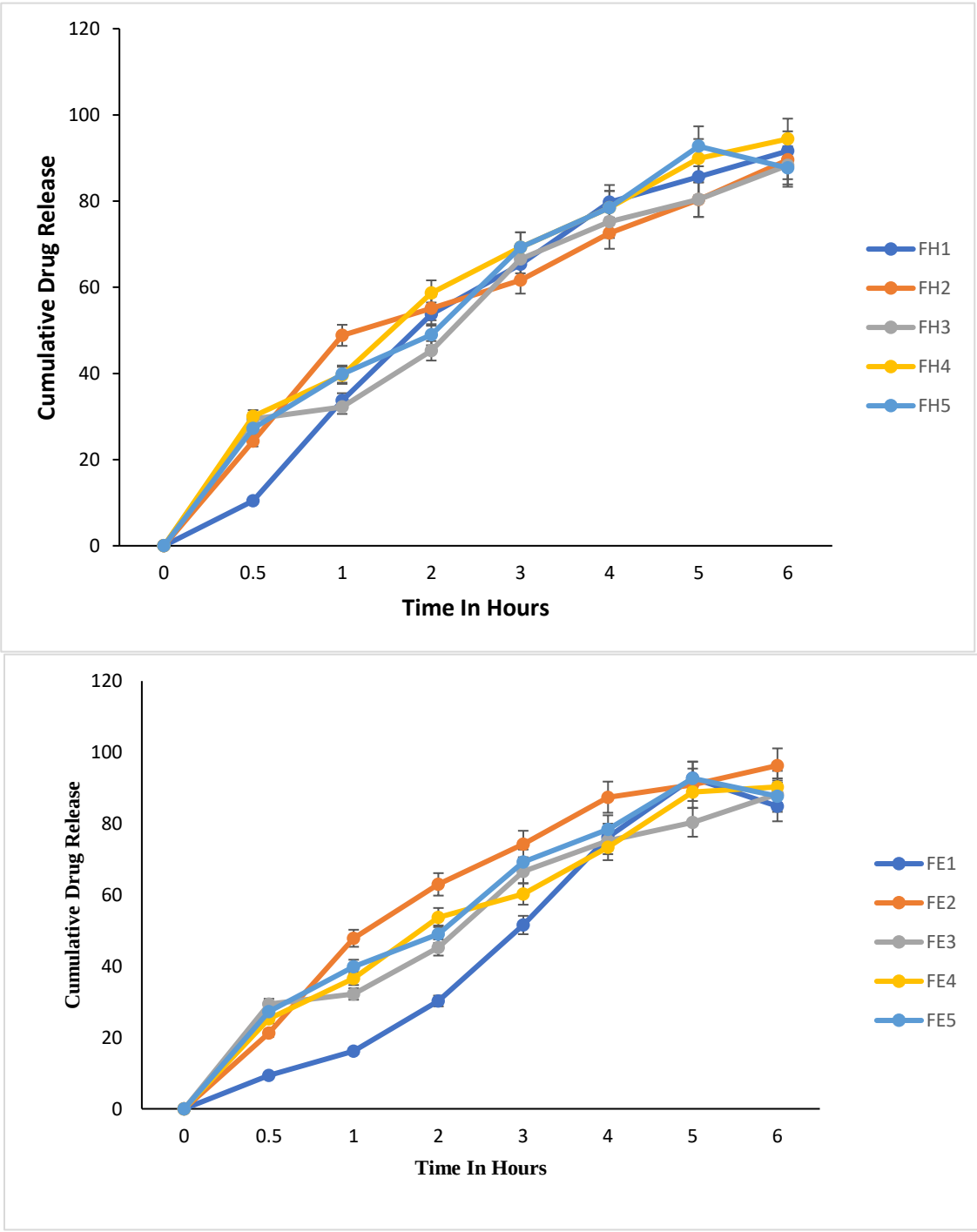


Figure No. 6. The drug release profile results in Figures provide insights into the release kinetics and sustained release potential of the phospholipid complex formulations of both the extracts loaded formulations of *Helicteres isora*.

3.7. Stability Study

Stability studies of optimized *H. isora* ethanolic extract loaded phytophospholipid complexes(FE2) and H. isora hydroalcoholic extract loaded phytophospholipid complexes(FH4) were conducted according to ICH guidelines (Q1AR2). The formulations were stored under different conditions: room temperature, accelerated temperature (25±2ºC, 60±5% RH), and (40±2ºC, 75±5% RH) for 6 months. At specified intervals, samples were analyzed in triplicates for average particle size, zeta potential, and entrapment efficiency to assess their stability(Table No6).

Table No.6. Stability studies of Optimized Phytosomal Formulations (FE2 & FH4)

Parameters	**Initial Values (0 month)**	**1 month**		**3 month**		**6 month**	
		(40±2ºC,75±5 %RH)	(25±2ºC, 60±5%RH)	(40±2ºC,75±5 %RH)	(25±2ºC, 60±5%RH)	(40±2ºC,75±5 %RH)	(25±2ºC, 60±5%RH)
Mean Particle Size (in nm)	198.8 ± 2.03	195.4 ± 1.25	196.6± 1.27	196.4 ± 0.34	197.9 ± 3.20	200.2± 2.16	199.2 ± 1.42
% EE	75.89 ± 2.30	74.67±0.26	73.339±2.10	72.89±0.12	69.13±0.19	67.01±0.37	65.06±1.29

Parameters	**Initial Values (0 month)**	**1 month**		**3 month**		**6 month**	
		(40±2ºC,75±5 %RH)	(25±2ºC, 60±5%RH)	(40±2ºC,75±5 %RH)	(25±2ºC, 60±5%RH)	(40±2ºC,75±5 %RH)	(25±2ºC, 60±5%RH)
Mean Particle	200.57± 2.53	200.8 ± 1.05	200.34± 1.14	205.4 ± 0.81	202.34 ± 2.50	208.2± 3.11	204.7 ± 1.39

Size (in nm)							
% EE	75.48 ± 3.2	73.41±0.15	73.23±1.10	71.89±0.12	71.13±0.11	71.01±0.24	69.06±0.19

4. Preparation and Characterization of Phytophospholipid Complex loaded Gel Formulations

The required amount of methyl and propyl paraben were dissolved in 10 ml of distilled water in a beaker to which Carbopol® 934P was dispersed and stirred continuously. The solution of RA/ FE2/FH4 was prepared in 0.1 ml of ethanol in another beaker and was added to the Carbopol dispersion. PEG 400 was also added to the dispersion. Triethanolamine was added to the dispersion to form gels. Prepared gels were filled in Al-tubes and stored at room temperature for further studies.

4.1. Characterization of the Phytophospholipid Complex loaded Gel Formulations:

The Prepared Phytophospholipid Complex loaded Gel Formulations were evaluated for various parameters like pH, Spreadability, viscosity, Uniformity and physical appearance.

pH: The Prepared gel formulation pH was determined by using a digital pH meter. 1 gm of gel was dispersed in 10 ml of distilled water and left for 2 hours. In three separate measurements, the pH of formulation was determined, and the average values were reported (15). The pH of the gel formulations was found to be in the range of 6.2 to 5.90±0.5, while that of blank gel was found to be 7 As the measured pH complies with the skin pH which 6.5 to 6.8 the prepared formulation would do not exhibit any irritation (Table No.7).

4.2 Spreadability

The therapeutic effect of the phytophospholipid Complex loaded Gel Formulations depends on the spreading value. The spreadibility of FE2G2 gel was 6.6±2.6 cm and of FH4G2 was 6.8±2.3cm, as compared to standard drug which was 6.2±1.9cm. Viscosity and Uniformity results are also represented in the table and the gel formulations were found stable(Table No.7).

Table No. 7. Evaluation of Phytophospholipid Complex loaded Gel Formulations

Formulation Code	pH	Spreadability Increase in Diameter (cm)	Viscosity (Cps)	Uniformity	Physical Appearance
RAG1	6.78±0.045	6.2±1.9	5180±10.4	Good	Light Yellow, Greasy, Homogenous
RAG2	6.77±0.21	5.6±2. 11	5370±11.8	Good	Light Yellow, Greasy, Homogenous
FE2G1	5.90±0.019	6.2±1.2	5690± 15.6	Good	Light Yellow, Greasy, Homogenous
FE2G2	6.2±0.016	6.6±2.6	5500± 21.7	Good	Light Yellow, Greasy, Homogenous
FH4G1	6.68±0.29	6.5±2.02	5705± 24.6	Good	Light Yellow, Greasy, Homogenous
FH4G2	6.9±0.27	6.8±2.3	5803± 22.4	Good	Light Yellow, Greasy, Homogenous

Transmission electron microscopy (TEM) Study

Transmission electron microscopy (TEM) was used to characterize the morphology of FE2G1, FE G2, FH4G1, and FH4G2 at an accelerating voltage of 100 kV (Figure 6). After diluting the lotion sample with distilled water, a drop of the sample was applied to a copper grid covered with carbon to create a thin layer, and a drop of 1% w/v phosphotungstic acid was added to negatively stain the sample. After letting the grid air dry, the samples were examined and captured on camera.

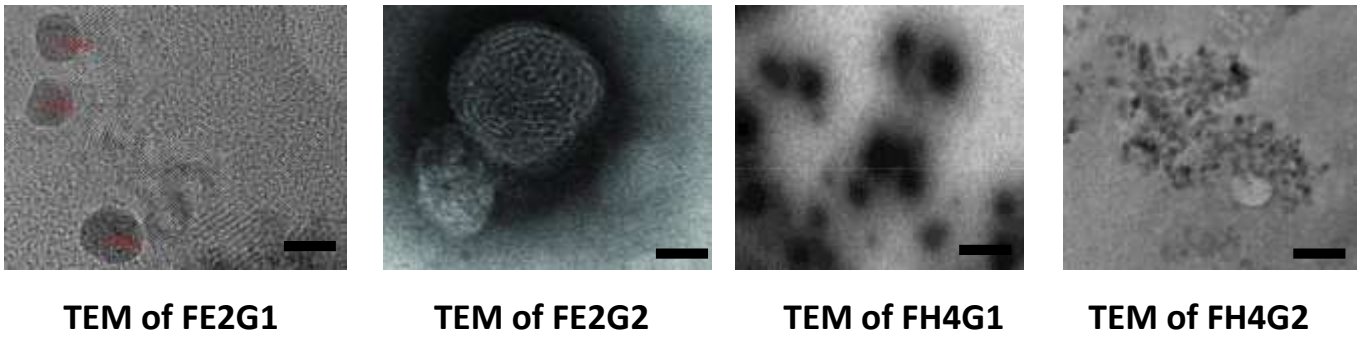


Figure No. 3.7. TEM of Phytophospholipid Complex loaded Gel Formulations

Skin penetration study of different formulations by Confocal Laser Microscopy [CLSM]

The skin penetration impact of all the produced formulations of (FE2G1, FE G2, FH4G1, and FH4G2) was investigated using goat skin in vitro utilizing confocal laser microscopy. Because goat skin is anatomically and physiologically comparable to human skin, it was used to test the skin-penetration capabilities of the novel formulations. Rhodamine 123 was used to measure the skin penetration of each of the four formulations. In short, the skin was homogeneously and non-occlusively treated with the test samples and the probe that contained 0.03% rhodamine. Franz diffusion cells were used for the studies, and a phosphate buffer pH 5.5 solution was placed in the receiver chamber. The skin was taken off and cleaned with phosphate buffer after a 24-hour period. After quickly freezing the skin with liquid nitrogen, a rectangular piece of skin perpendicular to the medication administration site was removed using a sharp blade. This tissue was adhered to the sample holder using a tissue frozen medium gel. (Gung, Germany; Leica). With the use of a cryomicrotome (Leica, Germany), pieces of skin that were perpendicular (dermis to horny layer) and 250 µm thick were cut. Probe penetration was examined after the treated region was taken off. Through the Z-axis of a Leica DMIRE2 confocal laser scanning microscope (Germany) connected to a Leica TCS SP2 fluorescent microscope, the whole thickness of the skin was optically scanned at intervals of 15–30 nm. The CLSM images are displayed below.

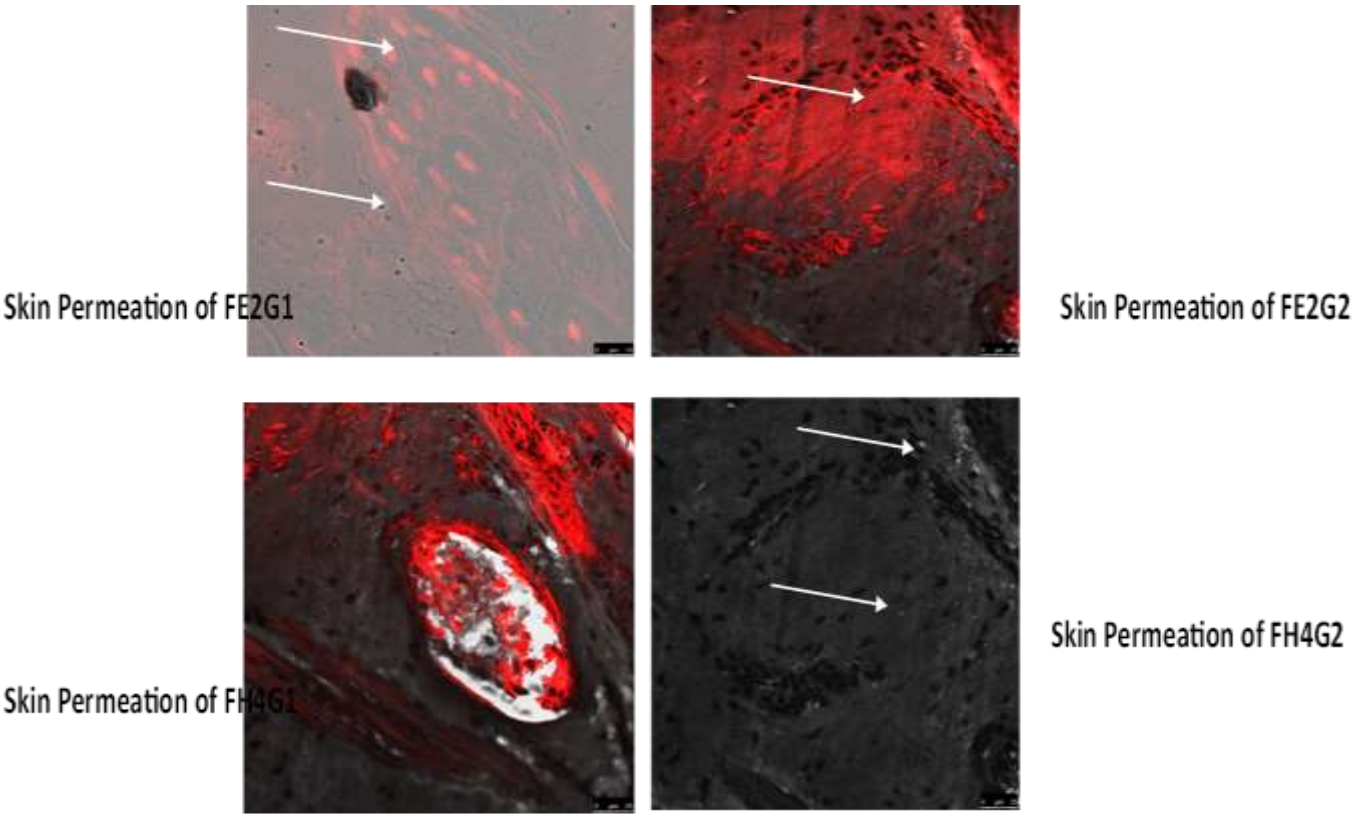


Figure No. 3.8. Skin penetration study of different formulations by Confocal Laser Microscopy [CLSM]

Through the above studies, skin penetrating effect of the novel formulations were studied using CLSM. The result showed that the phytophospholipid complexes embedded in to the gels have the ability to penetrate deeper in to the dermal layer and exert theirs effect. The enhanced penetration effect may be due to Phospholipids have a bilayer structure similar to the skin's natural lipid barrier. This allows phytophospholipid complexes to integrate more easily into the stratum corneum (the outermost layer of the skin), facilitating better penetration of the active ingredients. The similarity between the phospholipids and the skin's own lipids helps the bioactive compounds move through the skin layers more efficiently. Also, The lipid-loving properties of the phytophospholipid complex make it easier for the bioactive ingredients to diffuse through the lipid layers of the skin. In gel formulations, phytophospholipid complexes offered better retention and controlled release of the active ingredients. The slow and sustained release from the gel improved the overall penetration of the active compounds over time. This is proved particularly beneficial in H.isora

extract loaded topical treatments where prolonged exposure to the active ingredient was desired. Moreover, the percent drug retention of the formulation after 24 hours was satisfactory in comparison to the marketed formulation (Table No 3.8).

Table No. 3.8. Permeation Study of different phytophospholipid complex loaded gel formulations across abdominal goat skin.

Formulation	Jss (µg/ch^{-2}/h)	P (Ch/h)	LT (hr)	D^d (Ch^2/h)
RAG1	5.30±1.02	0.250±1.42	2.7	6.3
RAG2	5.68±0.59	0.280±1.2	2.4	6.7
FE2G1	5.44±1.34	0.280±1.64	2.0	7.2
FE2G2	6.65±0.46	0.238±1.56	1.6	8.6
FH4G1	6.23± 0.22	0. 198± 1.65	1.43	8.49
FH4G2	6.89± 0.46	0. 24± 1.46	1.55	8.79
Marketed Product (Capsaicin™ Herbal Gel)	5.57±0.63	0.220±1.59	2.0	7.7

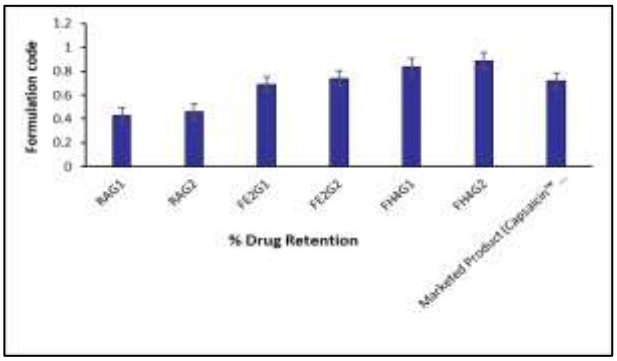
Where, Jss=transdermal flux, P=Permeability coefficient, LT=lag time, D^d =Diffusion coefficient

3.1.1. Skin Retention Study

A skin retention study of different formulations was conducted to determine how much rosmarinic acid was retained in the skin after 24 hours of diffusion. In this study, a higher percentage of drug was retained by FH4G2 formulations than other formulations. The % retention was near about similar for Marketed formulation, 0.720±30 and that of FE2G2 i.e. 0.78± 0.45.

Table No. 3.9. Percentage drug retention of different formulations in goat skin after 24 (h).

Formulation code	% Drug Retention
RAG1	0.43±1.23
RAG2	0.46±1.49
FE2G1	0.69±0.83
FE2G2	0.78± 0.45
FH4G1	0.82±0.23
FH4G2	0.54±0.19
Marketed Product (Capsaicin™ Herbal Gel)	0.720±30



3.1.2. Glutamate-induced paw licking and edema test:

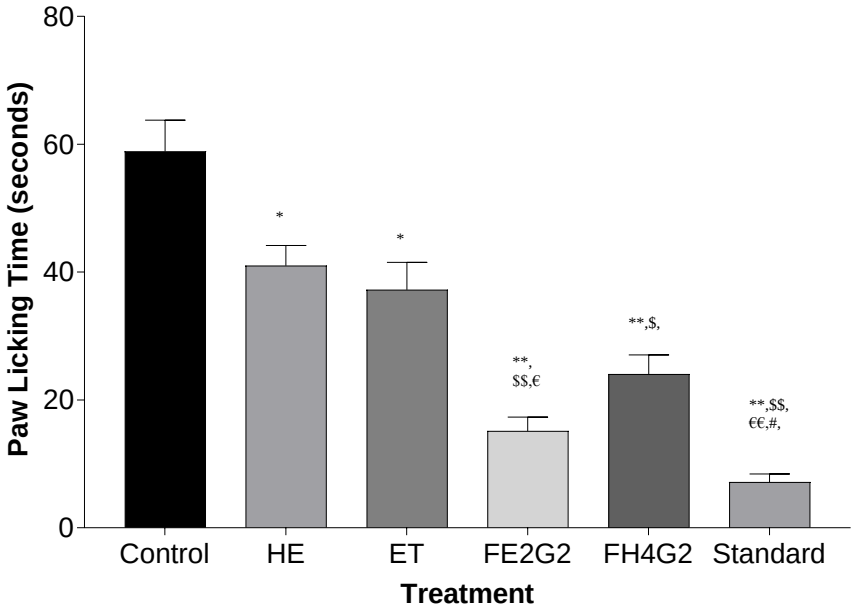


Figure No. 3.10 showing Glutamate-Induced Paw Licking and Edema Test. Data are expressed as Mean ± SEM (n=6). Data were analysed by using one-way ANOVA followed by Tukey's multiple comparison test. $^{*}P<0.01$ and $^{**}P<0.0001$ vs Control; $^{\$}P<0.05$ and $^{\$\$}P<0.0001$ vs HE; $^{€}P<0.05$ and $^{€€}P<0.0001$ vs ET; $^{\#}P<0.05$ vs FH4G2

The Glutamate-Induced Paw Licking and Edema Test is commonly used to evaluate the analgesic and anti-inflammatory properties of substances, including herbal formulations, in animal models. This test is based on the induction of nociceptive behavior (pain response) and inflammation by injecting glutamate into the paw of rodents. Mice were injected with 20 µl of 20 µ mol glutamate

(prepared in saline, pH 7.4) in the sub-plantar area of the right hind paw. Mice received vehicle, test samples and diclofenac sodium 15 min before glutamate injection respectively. The right hind paw thickness of mice was measured before glutamate challenge using vernier calliper. The licking was measured for 15 min after the glutamate challenge and accounted as indicative of nociception. The glutamate administration to mice significantly increased in the paw licking time (58.88 ± 4.9 s) in mice, and mice treated with extract HE and ET produced significant fall in paw licking time (41.01 ± 3.15s and 37.24 ± 4.3s, $P<0.01$ respectively). The formulation, FE2G2 and FH4G2 produced higher reduction paw licking duration (15.12 ± 2.19s and 24.02 ± 3.02s, respectively, $P<0.001$), while FE2G2 showed equal significance in decreasing the paw licking time as reduced by the administration of tramadol (7.16 ±1.25s).

CONCLUSION

The development of an effective herbal formulation using advanced techniques was successful in demonstrating the novel therapeutic properties of *Helicteres isora*. In this study, ethanolic and hydroalcoholic extracts from *H. isora* have been successfully extracted, characterized, and incorporated into phytophospholipid complexes in order to enhance their bioavailability and therapeutic effectiveness. The preparation of phytophospholipid complexes by modified solvent evaporation was stable and efficient. Based on characterization of the phytophospholipid complexes, it was revealed that they had favorable physical properties, such as appropriate particle size and zeta potential. It is crucial to have these properties for effective drug delivery and stability. The study also demonstrated the successful preparation and evaluation of herbal extract-loaded phytophospholipid complexes gels. Suitable for topical application, the gels displayed desirable physical properties, pH, spreadability, viscosity, and uniformity. These studies showed that phytosomal formulations proved to be more permeable and retained in the skin, indicating their effectiveness as transdermal delivery agents. Additionally, studies demonstrating glutamate-induced paw licking demonstrated beneficial effects of *H. isora* phytosomal gels. Gel formulations further enhanced the skin penetration of phytophospholipid complexes of *H.isora* extracts. As compared with the standard drug, the formulations were equally or more effective when it came to reducing inflammation. The complexation of phytoconstituents with phospholipids improved their stability against environmental factors (e.g., oxidation or degradation), which is crucial for maintaining the efficacy of the bioactive compounds throughout the shelf life of the formulation.

It appears that *Helicteres isora* has substantial therapeutic benefits, as well as the potential for developing novel herbal formulations with enhanced bioavailability and efficacy. As a result of the findings, further research and potential clinical applications are possible for *H. isora* in the treatment of inflammatory conditions.

4. Conflict of Interest: Nil

5. Acknowledgement: The authors want to acknowledge the Management of the Rungta College of Pharmaceutical Sciences and Research, Bhilai, Chhattisgarh, for providing research facilities and e-resources for carrying out the research work.

6. References

1. Pohocha, N. & Grampurohit, D. (2001). Antispasmodic activity of the fruits of Helicteres isora Linn, Phytother Res.15 (1), pp-49-52.
2. Tambekar, D.H. , Khante, B. S., Panzade, B. K., Dahikar, S.B., & Banginwar, Ys.,(2008). Evaluation of phytochemical and antibacterial potential of Helicteres isora L. fruits against enteric bacterial pathogens, Afr J Tradit Complement Altern Med, 5(3), pp-290-302.
3. Shriram, Varsha, Jahagirdar, Sheetal, Latha, C., Kumar, Vinay, Dhakephalkar, Prashant, Rojatkar, Supada & Shitole, Mahadeo G, (2010).Antibacterial & antiplasmid activities of Helicteres isora L, Indian J Med Res,132,pp-94-97.
4. Mahire, S. P., & Patel, S. N., (2020.) Extraction of phytochemicals and study of its antimicrobial and antioxidant activity of Helicteres isora L., Clinical Phytoscience, pp-6-40
5. Didar, Zohreh, (2020). Comparative in vitro Study of the biological activity and chemical composition extracts of Helicteres isora L. obtained by water and subcritical water extraction, Food Quality and Safety, 4, pp-101–106.
6. Bilal Ahmad Bhat, Elanchezhiyan C & Sethupathy S, (2012), In vitro antioxidative role of Helicteres Isora (l), International Journal of Bioassays, , 01 (12), pp-177-183
7. Rattanamaneerusmee, Acharaporn, Thirapanmethee, Krit, Nakamura, Yasushi, Bongcheewin, Bhanubong & Traidej Chomnawang, Mullika, (2018). Chemopreventive and biological activities of Helicteres isora L. fruit extracts Research in Pharmaceutical Sciences, 13(6), pp-484-492

8. Kumar, G., Sharmila Banu, G., Murugesan, A.G. & Rajasekara Pandian, M., (2007). Effect of Helicteres isora. Bark Extracts on Brain Antioxidant Status and Lipid Peroxidation in Streptozotocin Diabetic Rats, Pharmaceutical Biology, 45:10, DOI: 10.1080/13880200701585782, pp-753-759.
9. Kumar G, Sharmila Banu G, Murugesan AG, Rajasekara Pandian M., (2006). Hypoglycaemic effect of Helicteres isora bark extracts in rats. J Ethnopharmacol, 107, pp- 304-307.
10. Qu WH, Li JG, Wang MS., (1991). Chemical studies on the Helicteres isora. J of China Pharm Univ, 22, pp- 203-6.
11. Kusumoto IT, Shimada I, Kakiuchi N, Hattori M, Namba T. & Supriyatna S., (1992). Inhibitory effects of Indonesian plant extracts on Reverse Transcriptase of an RNA Tumour Virus (I). Phytother Res., 6, pp-241-4.
12. Bean MF, Antoun M, Abramson D, Chang CJ, Mc Laughlin JL, Cassady JM. Cucurbitacin B and Isocucurbitacin B Cytotoxic components of Helicteres isora. J Nat Prod. 1985; 48:500-3.
13. Singh SB, Singh AK &Thakur RS. (1984). Chemical constituents of the leaves of Helicteres isora. Indian J Pharm Sci., 46, pp-148-9.
14. Dayal, Renu, Singh, Amrita, Ojha, Rudra P. & Mishra, K. P. (2015). Possible therapeutic potential of Helicteres isora (L.) and it's mechanism of action in diseases, Journal of Medicinal Plants Studies, 3(2), pp- 95-100
15. Bean MF, Antoun M, Abramson D, Chang CJ, McLaughlin JL, Cassady JM. Cucurbitacin B & isocucurbitacin B.(1985). Cytotoxic components of Helicteres isora. J Nat Prod, 48(3), pp-500-3.
16. Lee DH, Iwanski GB, Thoennissen NH., (2010). Cucurbitacin: ancient compound shedding new light on cancer treatment. The Scientific World Journal,10, pp-413-8.
17. Gupta RN, Pareek A, Suthar M, Rathore GS, Basniwal PK & Jain D, (2009). Study of glucose uptake activity of Helicteres isora Linn. Fruits in L-6 cell lines. International Journal of Diabetes in Developing Countries, 29(4), pp-170-3.
18. Venkatesh S, Reddy BM, Reddy GD, Mullangi R & Lakshman M. (2010). Antihyperglycemic and hypolipidemic effects of Helicteres isora roots in alloxan-induced diabetic rats: a possible mechanism of action. J Nat Med, 64(3), pp-295-304

19. Kumar V, Sharma M, Lemos M. & Shriram V.(2013). Efficacy of Helicteres isora L. against free radicals, lipid peroxidation, protein oxidation and DNA damage. Journal of Pharmacy Research, 6(6), pp-620-5.
20. Bhavsar SK, Föller M, Gu S, Vir S, Shah MB. & Bhutani KK., (2009). Involvement of the PI3K/AKT pathway in the hypoglycemic effects of saponins from Helicteres isora. J Ethnopharmacol, 126(3), pp-386-396.
21. Kumar G, Sharmila BG, Murugesan AG. & Rajasekara PM.,(2007). Effect of Helicteres isora bark extracts on brain antioxidant status and lipid peroxidation in streptozotocin diabetic rats. Pharmaceutical Biology, 45(10), pp-753-9.
22. Dhevi R., Gayathri K, Shabi MM, Subashini U, Dubey GP. & Rajamanickam GV., (2008). A preliminary biochemical screening of Helicteres isora L. stem bark in carbon tetrachloride induced toxicity in rats. Bulgarian Journal of Veterinary Medicine, 11(4), 235-242.
23. Chitra MS, Prema S., (2009). Hepatoprotective activity of Helicteres isora Linn. Against CCl4 induced hepatic damage in rats. Hamdard Medicus, 52(1), pp-112-5.
24. Pradhan M, Sribhuwaneswari S, Karthikeyan D, Minz S, Sure P. & Chandu AN., (2008) . In vitro cytoprotection activity of Foeniculum vulgare and Helicteres isora in cultured human blood lymphocytes and antitumour activity against B16F10 melanoma cell line. Research Journal of Pharmacy and Technology, 1(4), pp-450-2.
25. Bhat BA, Elachezhiyan C, Sethupathy S., (2012). In vitro antioxidative role of Helicteres isora. International Journal of Bioassays, 1(12), pp-177-83.
26. Raaman N & Balasubramanian K, (2012). Antioxidant and anticancer activity of Helicteres isora dried fruit solvent extracts. J Acad Indus Res, 1(3), pp-148-52.
27. Sandhya T, Lathika KM, Pandey BN, Bhilwade HN, Chaubey RC & Priyadarsini KI, (2006). Protection against radiation oxidative damage in mice by Triphala. Mutat Res/Genetic Toxicology and Environmental Mutagenesis, 609(1), pp-17-25.
28. Velho-Pereira R, Kumar A, Pandey BN, Jagtap AG & Mishra KP. (2011). Radiosensitization in human breast carcinoma cells by thymoquinone: role of cell cycle and apoptosis. Cell Biol Int, 35(10), pp-1025-9.
29. Alam, M.A., Subhan, N., Abdul, Awal. M,, Alam. M,S,, Sarder, M., Nahar, L.& Sarker, S.D., (2009). Antinociceptive and anti-inflammatory properties of Ruellia tuberosa. Pharm. Biol, 47, pp-209-214.

30. Badole, S.L., Zanwar, A.A., Ghule, A.E., Ghosh, P. & Bodhankar, S.L., (2012). Analgesic and anti-inflammatory activity of alcoholic extract of stem bark of Pongamia pinnata (L.) Pierre. Biomed. Aging Pathol, 2, pp-19-23.
31. Tibrewal, R. (2017). Anti-Diabetic & Antioxidant Studies of Helictere Isora Roots. International Journal of Pharmaceutical and Biological Science Archive, 5(1).
32. Sama Venkatesh, G. Dayanand Reddy & B. Madhava Reddy (2003). Antihyperglycemic Activity of Helicteres isora Roots in Alloxan-Induced Diabetic Rats, Pharmaceutical Biology, 41:5, pp-347-350
33. G. Kumar, G. Sharmila Banu, AG Murugesan. (2009). Attenuation of Helicteres isora L. bark extracts on streptozotocin-induced alterations in glycogen and carbohydrate metabolism in albino rats. Human & Experimental Toxicology, 28(11), pp-689-696
34. Amita Jain, Rashmi Ranade, Prem Pritam, Neelu Joshi, Sirisha Lakshmi Vavilala, Ankita Jain,(2014) A Comparative Study of Antioxidant Activity, Total Phenolic and Flavonoid Contents in Different Parts of Helicteres isora L., American Journal of Life Sciences.2(5),pp. 292-302.
35. Padmanabh B. Deshpande et al.(2019) Determination and Fingerprinting of Gallic Acid in Hydroalcoholic Extract of Helicterus Isora (Malvaceae) By HPTLC Method., Indo Am. J. P. Sci, 06 (04),pp- 8503-8508
36. Nataraj Loganayaki, Perumal Siddhuraju & Sellamuthu Manian (2011). Antioxidant activity and free radical scavenging capacity of phenolic extracts from Helicteres isora L. and Ceiba pentandra L. Journal of Food Science and Technology volume 50, pp-687–695
37. Govindasami C, Chakkaravarthy E, Ghosh K. (2016). Determination of antidiabetic compound from Helicteres isora by oral glucose tolerance test. J App Pharm Sci, 6 (02):pp- 172-174
38. Veena Sharma*. , Urmila Chaudhary. (2016). Pharmacognostic And Phytochemical Screening of *Helicteres Isora* Roots. Asian J Pharm Clin Res., 9(2),pp-96-101
39. Sanjeet Kumar, Padan Kumar Jena, Monika Kumari, Navyanita Patnaik, Ashok Kumar Nayak and Prakash Kumar Tripathy. (2013), Validation of tribal claims through pharmacological studies of Helicteres isora L. leaf extracts: an Empirical Research., Int. J. Drug Dev. & Res., 5(1) pp- 279-285.
40. Mahesh Bandappa Manke , Shashikant Chaburao Dhawale, Prasad Govindrao. (2015). Asian Pac J Trop Dis., 5(4): pp-313-315

41. Hota S, & Chatterjee A. (2016). Traditional and indigenous uses of plants for treatment of skin diseases by the tribes in Paschim Medinipur district of West Bengal. Journal of Medicinal Plant Studies, 4(5), pp- 175-180
42. Jha AK, & Gurudatta Y.(2015). Some Wild Trees of Bihar and Their Ethnobotanical Study. Journal of Research & Method in Education, 5 (6 [Ver. II]) , pp-74-76.
43. Sivaranjani R & Ramakrishnan K. (2012). Traditional uses of medicinal plants in treating skin diseases in Nagapattinam district of Tamilnadu, India. International Research Journal of Pharmacy, 3(5), pp-201-204.
44. Azaizeh H, Fulder S, Khalil K & Said Q. (2003), Ethnomedicinal knowledge of local Aeab practioners in the middle East Region. Fitoterapia, 74, pp-98-108.
45. Newman DJ & Cragg GM. (2007). Natural products as source of new drugs over the last 25 years. J Natl Prod., 70, pp-461-77.
46. Rahaman CH, Ghosh A & Mondal S.(2008). Studies on ethnomedicinal uses of plants by the tribals of Birbhum district, West Bengal. Ind J Environ Ecoplan, 15, pp-71-78.
47. Rollinger JM, Langer T. & Stuppner H. (2006). Strategies for efficient lead structure discovery from natural products. Curr Med Chem., 13, pp-1491.
48. Gupta A, Mishra AK, Bansal P, Kumar S, Sannd R, Gupta V, Goyal BM, Singh AK & Kumar A. (2010), Antileprotic Potential of Ethnomedicinal Herbs: A Review. Drug Invention Today, 2(3), pp-191-193.
49. Susmitha S, Vidyamol KK, Ranganayaki P, Vijayaragavan R. (2013). Phytochemical Extraction and Antimicrobial Properties of Azadirachta indica (Neem). Global Journal of Pharmacology, 7 (3), pp- 316-320.